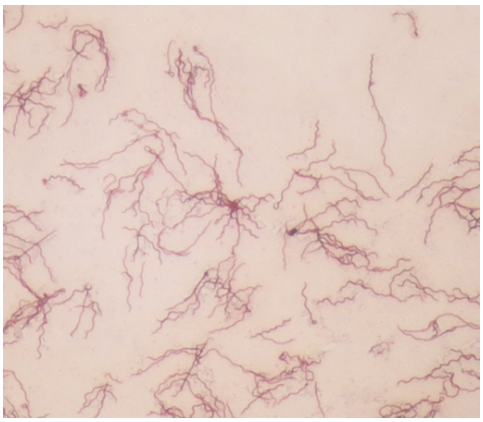
MAINE MOBILE BIOLAB
STUDENT HANDOUT

Name:	

Parasite Predicament

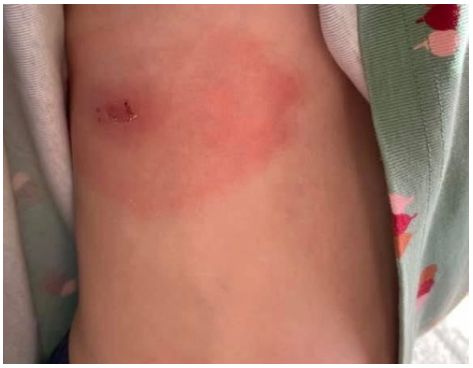
Diagnosing and Investigating the Transmission of an Infectious Disease



A patient that arrives at the ER is a 34-year-old elementary school teacher who enjoys hiking and spending time outdoors. She lives in a wooded, rural area in Maine and frequently walks her dog on nearby trails. She has no significant past medical history, takes no daily medications, and has no known allergies.

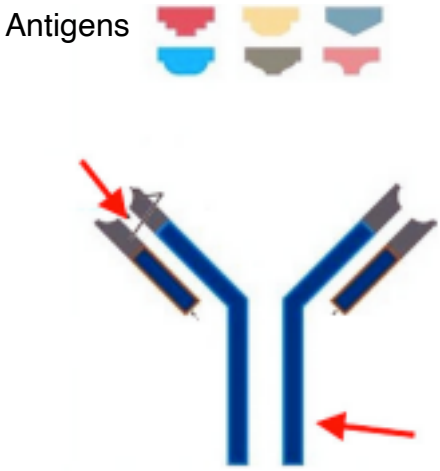
Approximately two weeks ago, she went on a weekend camping trip, and she recalls removing a small tick from her left calf a few days after returning, but did not think much of it at the time. Over the past week, she has developed increasing fatigue, mild headaches, and intermittent joint pain, particularly in her knees and wrists. Yesterday, she noticed a large, circular rash with a central clearing on her left calf, which has since expanded. Concerned about her symptoms, she decided to come to the emergency department.

The patient appears fatigued but is alert and oriented. She describes a dull headache, mild neck stiffness, and muscle aches. She denies fever, chills, nausea, vomiting, or neurological symptoms like numbness or weakness. She reports mild joint swelling, but no redness or warmth.



Given her history of a tick bite, recent outdoor exposure, and the presence of erythema migrans (a skin rash that is caused by the bacterium *Borrelia burgdorferi*), Lyme disease is highly suspected. To test this patient for Lyme disease, you will use an enzyme-linked immunosorbent assay (ELISA), which will demonstrate the presence of antibodies to the *B. burgdorferi* bacteria with a color change. An ELISA tests for the presence of a specific antigen for a particular antibody. Antigens serve as the target for the receptors of an immune response. Antibodies are large Y-shaped proteins that identify and neutralize pathogens.

Label the antibody and receptor site on the diagram. Which antigen is going to bind to the antibody?

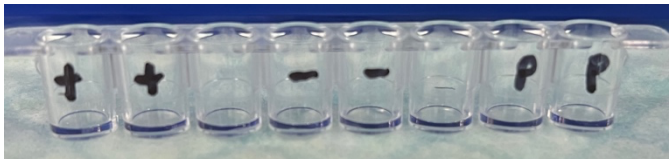


MATERIALS

- Positive control
- Negative control
- Patient's sample
- Primary Antibody
- Secondary antibody
- Enzyme substrate
- 8-well coated microplate strip
- Micropipettes
- Micropipette tips
- Wash buffer (saline and Tween)

PART 1 – Microplate Preparation

1. Label the first two wells of your microplate strip with "+" for the positive control, then leave the next one blank. Label the next two wells with a "-" for the negative control, then leave the next one blank. Label the final two wells with "p," for your patient's sample.



Sample	Tube Label	Well Numbers
Positive Control		
Negative Control		
Patient's Sample		

2. Using the P50 micropipette, transfer 50 µL of positive control into the designated wells.
3. With a new micropipette tip, transfer 50 µL of negative control into the designated wells.
4. With a new micropipette tip, transfer 50 µL of the patient's sample into the designated wells.
5. Wait 5 minutes to allow the proteins to bind to the wells.

QUICK CHECK: What does the positive control contain that the negative control does not?

WASHING THE BUFFER

6. Tip the microplate strip upside down on the provided stack of paper towels. Gently tap the bottom of the strip without lifting it. Remove the top paper towel when finished.
7. Using the P1000 micropipette, add 350 µL of wash buffer to each well. Be careful not to spill into neighboring wells.
8. Empty the contents onto paper towels. Tap gently without lifting (see figure below). Remove the top paper towel.

   Repeat steps 6-8.

ANTIBODY ADDITION

9. Use the P50 micropipette and a new tip to add 50 µL of Primary Antibody (PA) into all wells.
10. Wait 5 minutes for the primary antibodies to bind to their targets.

QUICK CHECK: What is the antigen-antibody relationship?

11. Wash the wells (steps 6-8) **two times**.
12. Use a new micropipette tip to transfer 50 µL of secondary antibody (SA) into all wells.
13. Wait 5 minutes for the secondary antibodies to bind to their targets.

QUICK CHECK: What is the importance of washing the wells (specifically with Tween)?

QUICK CHECK: Why is it important to wash the wells in between the addition of the primary and secondary antibodies?

14. Wash the wells (steps 6-8) **three times.**
15. With a new micropipette tip, add 50 µL of enzyme substrate (SUB) to each well.
16. Wait 5 minutes. Positive samples will begin to turn blue.

PART 2 – Results and Analysis

What does a positive ELISA result indicate about the patient's infection status?

Why might a patient with an early Lyme disease infection test negative using ELISA?